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(FILE 'HOME' ENTERED AT 18:53:10 ON 14 AUG 2001)

FILE 'CAOLD, CAPLUS, CROPU, DGENE, DPCI, ENCOMPPAT, ENCOMPPAT2,
EUROPATFULL, IFIPAT, INPADOC, JAPIO, PAPERCHEM2, PATDD, PATDPA, PATOSDE,
PATOSEP, PATOSWO, PCTFULL, PIRA, RAPRA, SYNTHLINE, TULSA, TULSA2,
USPATFULL, WPIDS' ENTERED AT 18:53:19 ON 14 AUG 2001

L1 870560 S (SACCHARIDE# OR MONOSACCHARIDE# OR OLIGOSACCHARIDE# OR CARBOH
L2 383649 S L1 (L) (PREPAR? OR MAK? OR SYNTH? OR CONV? OR PREP/RL)
L3 5905 S GLYCOSYLTRANSFERASE#
L4 453 S L3 (L) (FUSION PROTEIN# OR CHIMER? PROTEIN# OR (PROTEINS (L)
L5 402 S L2 (L) L4
L6 396 DUP REM L5 (6 DUPLICATES REMOVED)
L7 108 S L6 AND PY<=1997
L8 23 S L7 AND (SUGAR# (W) NUCLEOTIDE#)
L9 10 S L8 AND (REGENER? OR RECYCL?)

=> s l9 and (plant? or microorganism?)

8 FILES SEARCHED...

21 FILES SEARCHED...

L10 9 L9 AND (PLANT? OR MICROORGANISM?)

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L10 ANSWER 1 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent
ACCESSION NUMBER: 1995024495 PCTFULL
TITLE (ENGLISH): TRANSGENIC PRODUCTION OF OLIGOSACCHARIDES
AND GLYCOCONJUGATES
TITLE (FRENCH): PRODUCTION TRANSGENIQUE D'OLIGOSACCHARIDES
ET DE GLYCOCONJUGUES
INVENTOR(S): PRIETO, Pedro, Antonio; SMITH, David, Fletcher;
CUMMINGS, Richard, Dale; KOPCHIK, John, Joseph;
MUKERJI, Pradip; MOREMEN, Kelley, Wilson; PIERCE,
James, Michael
PATENT ASSIGNEE(S): ABBOTT LABORATORIES
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

| NUMBER | KIND | DATE |
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|--------|------|------|

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| WO 9524495 | A1 | 19950914 |
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| DESIGNATED STATES: | AU CA FI JP MX NL NZ AT BE CH DE DK ES FR GB GR IE IT |
| | LU MC NL PT SE |

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| APPLICATION INFO.: | WO 1995-US967 | 19950124 |
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| PRIORITY (ORIGINAL): | US 1994-208889 | 19940309 |
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ABEN The invention relates to the milk of a transgenic non-human mammal. The milk is characterized in that it contains heterologous components produced as the secondary gene products of a heterologous gene contained in the genome of the transgenic non-human mammal. The heterologous gene encodes a heterologous catalytic entity such as a human enzyme selected from the group consisting of **glycosyltransferases**, phosphorylases, hydroxylases, peptidases and sulfotransferases. Especially useful in the practice of the invention are human **glycosyltransferases**. The desired heterologous components include **oligosaccharides**, glycoconjugates. The **oligosaccharides** and glycoconjugates may be isolated from the milk of the transgenic mammals and used in the **preparation** of pharmaceuticals, diagnostic kits, nutritional products and the like. The whole transgenic milk may also be used to formulate nutritional products that provide special advantages. The transgenic milk may also be used in the production of specialized enteral nutritional products.

ABF L'invention se rapporte au lait d'un mammifere transgenique autre que l'homme. Ce lait se caracterise en ce qu'il contient des composants heterologues obtenus sous forme de produits geniques secondaires d'un gene heterologue contenu dans le genome du mammifere transgenique. Le gene heterologue code une entite catalytique heterologue, telle qu'une enzyme humaine selectionnee dans le groupe compose de **glycosyltransferases**, phosphorylases, hydroxylases, peptidases, et sulfotransferases. Des **glycosyltransferases** humaines sont notamment utiles dans la mise en application de l'invention. Les composants heterologues desirés comprennent des **oligosaccharides** et des glycoconjugues. Les **oligosaccharides** et les glycoconjugues peuvent etre isolés du lait des mammiferes transgeniques et utilises dans la **preparation** de produits pharmaceutiques, de kits diagnostiques, de produits nutritionnels et analogues. Le lait entier transgenique peut etre egalement utilise pour formuler des produits nutritionnels qui presentent des avantages specifiques. Ce lait transgenique peut aussi etre utilise dans la production de produits nutritionnels enteriques

specialises.

L10 ANSWER 2 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent
ACCESSION NUMBER: 1995024494 PCTFULL
TITLE (ENGLISH): HUMANIZED MILK
TITLE (FRENCH): LAIT HUMANISE
INVENTOR(S): PRIETO, Pedro, Antonio; SMITH, David, Fletcher;
CUMMINGS, Richard, Dale; KOPCHIK, John, Joseph;
MUKERJI, Pradip; MOREMAN, Kelley, Wilson; PIERCE,
James, Michael
PATENT ASSIGNEE(S): ABBOTT LABORATORIES
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

| NUMBER | KIND | DATE |
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| WO 9524494 | A1 19950914 | |
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| DESIGNATED STATES: | AU CA FI JP MX NO NZ AT BE CH DE DK ES FR GB GR IE IT |
| | LU MC NL PT SE |
| APPLICATION INFO.: | WO 1995-US926 19950124 |
| PRIORITY (ORIGINAL): | US 1994-209122 19940309 |

ABEN The invention relates to humanized milk. The milk is produced by a non-human transgenic mammal wherein the genome of said transgenic non-human mammal contains at least one heterologous gene encoding for a human catalytic entity and wherein the catalytic entity produces **oligosaccharides** and glycoconjugates that are present in the milk of

said transgenic non-human mammal. An especially useful catalytic entity is human **glycosyltransferases** which produce

oligosaccharides and

glycoconjugates. A method of obtaining humanized milk is disclosed. The method comprises the steps of (a) inserting into the genome of a non-human mammal a heterologous gene encoding the production of a human catalytic entity wherein said catalytic entity produces a secondary gene product in the milk of said non-human mammal; and (b) milking said non-human mammal. The humanized milk may be used in the **preparation**

of an enteral nutritional product useful in the nutritive maintenance of an animal.

ABF L'invention concerne du lait humanise. Le lait est produit par un mammifere transgenique non humain, le genome de ce dernier contenant au moins un gene heterologue codant pour une entite catalytique humaine, laquelle produit les **oligosaccharides** et les glycoconjugues presents

dans le lait dudit mammifere. L'entite catalytique particulierement utile est constituee de **glycosyltransferases** humaines produisant des

oligosaccharides et des glycoconjugues. L'invention concerne egalement

un procede de production de lait humanise qui consiste a: (a) introduire dans le genome d'un mammifere non humain un gene heterologue codant la production d'une entite catalytique humaine, laquelle engendre un produit genique secondaire dans le lait dudit mammifere non humain; et (b) a traire ledit mammifere non humain. Ce lait humanise peut etre utilise dans la **preparation** d'un produit nutritif enteral utile dans

l'alimentation d'un animal.

L10 ANSWER 3 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent
ACCESSION NUMBER: 1995024488 PCTFULL
TITLE (ENGLISH): TRANSGENIC ANIMALS PRODUCING OLIGOSACCHARIDES
AND GLYCOCONJUGATES
TITLE (FRENCH): ANIMAUX TRANSGENIQUES PRODUISANT DES
OLIGOSACCHARIDES ET DES

INVENTOR(S): GLYCOCONJUGUES
 PRIETO, Pedro, Antonio; SMITH, David, Fletcher;
 CUMMINGS, Richard, Dale; KOPCHIK, John, Joseph;
 MUKERJI, Pradip; MOREMEN, Kelley, Wilson; PIERCE,
 James, Michael
 PATENT ASSIGNEE(S): ABBOTT LABORATORIES
 LANGUAGE OF PUBL.: English
 DOCUMENT TYPE: Patent
 PATENT INFORMATION:

| NUMBER | KIND | DATE |
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|--------------------|---|-------------|
| DESIGNATED STATES: | WO 9524488 | A1 19950914 |
| | AU CA FI JP MX NO NZ AT BE CH DE DK ES FR GB GR IE IT | |
| | LU MC NL PT SE | |

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| APPLICATION INFO.: | WO 1995-US1147 | 19950124 |
| PRIORITY (ORIGINAL): | US 1994-209132 | 19940309 |

ABEN The invention relates to transgenic non-human mammals characterized in that the genome of said mammals contain at least one heterologous gene encoding for the production of heterologous catalytic entity selected from the group consisting of enzymes and antibodies, and wherein said catalytic entity produces a second heterologous product in the milk of said mammal. Especially useful in the practise of the invention are human **glycosyltransferases** and transgenic sheep, goats and cows. The heterologous product includes **oligosaccharides** and glycoconjugates.

ABF L'invention se rapporte a des mammiferes transgeniques autres que l'homme qui se caracterisent en ce que leur genome contient au moins un gene heterologue codant pour la production d'une entite catalytique heterologue selectionnee dans le groupe compose d'enzymes et d'anticorps, cette entite catalytique formant un second produit heterologue dans le lait du mammifere. Les **glycosyltransferases** humaines ainsi que les moutons, les chevres et les vaches transgeniques sont particulierement utiles dans la mise en application de l'invention. Le produit heterologue comprend des **oligosaccharides** et des glycoconjugues.

L10 ANSWER 4 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent
 ACCESSION NUMBER: 1993008205 PCTFULL
 TITLE (ENGLISH): PRODUCTION OF FUCOSYLATED CARBOHYDRATES BY
 ENZYMATIC FUCOSYLATION

SYNTHESIS OF SUGAR
 NUCLEOTIDES; AND IN SITU REGENERATION
 OF GDP-FUCOSE

TITLE (FRENCH): PREPARATION D'HYDRATES DE CARBONE FUCOSYLES
 PAR SYNTHESE A
 FLUCOSYLATION ENZYMATIQUE DE NUCLEOTIDES DE SUCRES, ET
 REGENERATION IN
 SITU DE GDP-FUCOSE

INVENTOR(S): WONG, Chi-Huey; ICHIKAWA, Yoshitaka; SHEN, Gwo-Jenn;
 LIU, Kun-Chin

PATENT ASSIGNEE(S): THE SCRIPPS RESEARCH INSTITUTE
 LANGUAGE OF PUBL.: English
 DOCUMENT TYPE: Patent
 PATENT INFORMATION:

| NUMBER | KIND | DATE |
|--------|------|------|
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|----------------------|---|-------------|
| DESIGNATED STATES: | WO 9308205 | A1 19930429 |
| | AU BB BG BR CA CS FI HU JP KP KR LK MG MN MW NO PL RO | |
| | RU SD AT BE CH DE FR GB GR IE IT LU MC NL SE BF BJ CF | |
| | CG CI CM GA GN ML MR SN TD TG | |
| APPLICATION INFO.: | WO 1992-US8789 | 19921015 |
| PRIORITY (ORIGINAL): | US 1991-777662 | 19911015 |

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| US 1992-901260 | 19920619 |
| US 1992-910612 | 19920708 |
| US 1992-961076 | 19921014 |

ABEN This invention contemplates improved methods of enzymatic production of **carbohydrates** especially fucosylated **carbohydrates**. Improved **syntheses** of glycosyl 1- or 2-phosphates using both chemical and enzymatic means are also contemplated. The phosphorylated glycosides are then used to produce **sugar nucleotides** that are in turn used as donor **sugars** for glycosylation of acceptor **carbohydrates**. Especially preferred herein in the use of a disclosed method for fucosylation.

ABF L'invention se rapporte a des procedes de production enzymatique d'hydrates de carbone, en particulier des hydrates de carbone fucosyles. Des **syntheses** ameliorees de 1- ou 2-phosphates de glycosyle, effectuees a la fois par l'intermediaire de moyens enzymatiques et de moyens chimiques sont egalement decrits. Les glucosides phosphoryles sont ensuite utilises pour produire des nucleotides de sucres qui sont a leur tour utilises comme sucres donneurs pour la glycosylation d'hydrates de carbone accepteurs. L'utilisation d'un procede de fucosylation decrit est particulierement preferee.

L10 ANSWER 5 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent
 ACCESSION NUMBER: 1992016640 PCTFULL
 TITLE (ENGLISH): **OLIGOSACCHARIDE ENZYME SUBSTRATES AND INHIBITORS: METHODS AND COMPOSITIONS**
 TITLE (FRENCH): **OLIGOSACCHARIDES SERVANT DE SUBSTRATS ET D'INHIBITEURS D'ENZYMES: PROCEDES ET COMPOSITIONS**
 INVENTOR(S): WONG, Chi-Huey; ICHIKAWA, Yoshitaka; SHEN, Gwo-Jenn
 PATENT ASSIGNEE(S): THE SCRIPPS RESEARCH INSTITUTE
 LANGUAGE OF PUBL.: English
 DOCUMENT TYPE: Patent
 PATENT INFORMATION:

| NUMBER | KIND | DATE |
|------------|------|----------|
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| WO 9216640 | A1 | 19921001 |

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| DESIGNATED STATES: | AT AU BE CA CH DE DK ES FR GB GR IT JP LU MC NL SE |
| APPLICATION INFO.: | WO 1992-US2178 19920317 |
| PRIORITY (ORIGINAL): | US 1991-670701 19910318 |
| | US 1991-707600 19910520 |
| | US 1991-738211 19910730 |
| | US 1992-852409 19920316 |

ABEN **Oligosaccharide** compounds that are substrates and inhibitors of **glycosyltransferase** and glycosidase enzymes and compositions containing such compounds are disclosed. A method of glycosylation is also disclosed. An E. coli transformed with phagemid CMPSIL-1, which phagemid comprises a gene for a modified CMP-sialic acid **synthetase** enzyme, which transformed E. coli has the ATCC accession No. 68531 is also provided.

ABF Composes **oligosaccharides** constituant des substrats et des inhibiteurs des enzymes **glycosyltransferase** et glycosidase et composition contenant ces composes. On decrit egalement un procede de glycosylation, ainsi qu'un E. coli transforme avec du phagemide CMPSIL-1, lequel phagemide comprend un gene pour l'enzyme **synthetase** d'acide CMP-sialique modifiee, l'E. coli transforme ayant recu le numero d'enregistrement ATCC 68531.

L10 ANSWER 6 OF 9 PCTFULL COPYRIGHT 2001 MicroPatent
 ACCESSION NUMBER: 1991013988 PCTFULL
 TITLE (ENGLISH): RECOMBINANT beta-1,4 GLUCAN **SYNTHASE**
 PROTEINS
 TITLE (FRENCH): PROTEINES RECOMBINANTES DE beta-1,4 GLUCANE
SYNTHASE
 INVENTOR(S): SAXENA, Inder, M., Jr.; LIN, Fong, Chyr; BROWN, R.,
 Malcolm, Jr.
 PATENT ASSIGNEE(S): THE BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM;
 SAXENA, Inder, M., Jr.; LIN, Fong, Chyr; BROWN, R.,
 Malcolm, Jr.
 LANGUAGE OF PUBL.: English
 DOCUMENT TYPE: Patent
 PATENT INFORMATION:

| NUMBER | KIND | DATE |
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| WO 9113988 | A1 | 19910919 |
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DESIGNATED STATES: AT AT AU BB BE BF BG BJ BR CA CF CG CH CH CM DE DE DK
 DK ES ES FI FR GA GR HU IT JP KP KR LK LU LU MC MG ML
 MR MW NL NL NO PL RO SD SE SE SN SU US

APPLICATION INFO.: WO 1991-US1726 19910314
 PRIORITY (ORIGINAL): US 1990-494093 19900315

ABEN The present invention relates to the compositions and methods
 associated with the cloning of the subunits of beta-1,4 glucan
synthases
 responsible for catalyzing beta-1,4 glucan polymer biosynthesis. The
 invention relates further to compositions and methods for obtaining host
 cells containing recombinant beta-1,4 glucan **synthases** as well
 as
 compositions and methods for obtaining beta-1,4 glucan **synthase**
 from
 natural sources. In certain aspects, the present invention provides
 methods for the cloning of both the 83 Kd subunit and the 93 Kd subunit
 of the cellulose **synthase** enzyme from Acetobacter xylinum.

ABF Compositions et procedes associes au clonage de sous-unites de
 beta-1,4 glucane **synthase** servant a catalyser la biosynthese du
 polymere
 beta-1,4 glucane. La presente invention a egalement trait a d'autres
 compositions et procedes permettant d'obtenir des cellules hotes
 contenant des beta-1,4 glucane **synthases** recombinantes et a des
 compositions et procedes permettant d'obtenir la beta-1,4 glucane
synthase de sources naturelles. Sous certains aspects, la
 presente
 invention presente des procedes pour le clonage tant de la sous-unite 83
 Kd et de la sous-unite 93 Kd de l'enzyme cellulose **synthase** a
 partir de
 Acetobacter xylinum.

L10 ANSWER 7 OF 9 USPATFULL
 ACCESSION NUMBER: 97:120488 USPATFULL
 TITLE: Methods of making transgenic animals producing
 oligosaccharides and glycoproteins
 INVENTOR(S): Prieto, Pedro Antonio, Columbus, OH, United States
 Smith, David Fletcher, Athens, GA, United States
 Cummings, Richard Dale, Edmond, OK, United States
 Kopchick, John Joseph, Athens, OH, United States
 Mukerji, Pradip, Gahanna, OH, United States
 Moremen, Kelley Wilson, Athens, GA, United States
 Pierce, James Michael, Athens, GA, United States
 PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States
 (U.S. corporation)

| NUMBER | KIND | DATE |
|--------|------|------|
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PATENT INFORMATION: US 5700671 19971223 <--
 APPLICATION INFO.: US 1995-434151 19950502 (8)
 RELATED APPLN. INFO.: Division of Ser. No. US 1994-209132, filed on 9 Mar 1994
 DOCUMENT TYPE: Utility
 FILE SEGMENT: Granted
 PRIMARY EXAMINER: Chambers, Jasemine C.
 ASSISTANT EXAMINER: Crouch, Deborah
 LEGAL REPRESENTATIVE: Becker, Cheryl L.
 NUMBER OF CLAIMS: 8
 EXEMPLARY CLAIM: 1
 NUMBER OF DRAWINGS: 19 Drawing Figure(s); 15 Drawing Page(s)
 LINE COUNT: 1805

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention relates to transgenic non-human mammals characterized in that the genome of said mammals contain at least one heterologous gene encoding for the production of heterologous catalytic entity selected from the group consisting of enzymes and antibodies, and wherein said catalytic entity produces a second heterologous product in the milk of said mammal. Especially useful in the practice of the invention are human glycosyltransferases and transgenic sheep, goats and cows. The heterologous product includes oligosaccharides and glycoconjugates.

L10 ANSWER 8 OF 9 USPATFULL

ACCESSION NUMBER: 97:3736 USPATFULL
 TITLE: Oligosaccharide enzyme substrates and inhibitors: methods and compositions
 INVENTOR(S): Wong, Chi-Huey, San Diego, CA, United States
 Ichikawa, Yoshitaka, San Diego, CA, United States
 Shen, Gwo-Jenn, Carlsbad, CA, United States
 PATENT ASSIGNEE(S): The Scripps Research Institute, La Jolla, CA, United States (U.S. corporation)

| | NUMBER | KIND | DATE | |
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| PATENT INFORMATION: | US 5593887 | | 19970114 | <-- |
| APPLICATION INFO.: | US 1995-476685 | | 19950607 | (8) |
| RELATED APPLN. INFO.: | Division of Ser. No. US 1994-219242, filed on 29 Mar 1994, now patented, Pat. No. US 5461143 which is a continuation-in-part of Ser. No. US 1992-852409, filed on 16 Mar 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-738211, filed on 30 Jul 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-670701, filed on 18 Mar 1991, now patented, Pat. No. US 5278299 And Ser. No. US 1991-707600, filed on 30 May 1991, now abandoned | | | |
| DOCUMENT TYPE: | Utility | | | |
| FILE SEGMENT: | Granted | | | |
| PRIMARY EXAMINER: | Fleisher, Mindy | | | |
| ASSISTANT EXAMINER: | Weiss, Bonnie D. | | | |
| LEGAL REPRESENTATIVE: | Welsh & Katz, Ltd. | | | |
| NUMBER OF CLAIMS: | 4 | | | |
| EXEMPLARY CLAIM: | 1 | | | |
| NUMBER OF DRAWINGS: | 3 Drawing Figure(s); 3 Drawing Page(s) | | | |
| LINE COUNT: | 3572 | | | |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Oligosaccharide compounds that are substrates and inhibitors of glycosyltransferase and glycosidase enzymes and compositions containing such compounds are disclosed. A method of glycosylation is also disclosed. An E. coli transformed with phagemid CMPSIL-1, which phagemid comprises a gene for a modified CMP-sialic acid synthetase enzyme, which transformed E. coli has the ATCC accession No. 68531 is also provided.

L10 ANSWER 9 OF 9 USPATFULL

ACCESSION NUMBER: 95:95009 USPATFULL
TITLE: Oligosaccharide enzyme substrates and inhibitors:
methods and compositions
INVENTOR(S): Wong, Chi-Huey, San Diego, CA, United States
Ichikawa, Yoshitaka, San Diego, CA, United States
Shen, Gwo-Jenn, Carlsbad, CA, United States
PATENT ASSIGNEE(S): The Scripps Research Institute, La Jolla, CA, United
States (U.S. corporation)

| | NUMBER | KIND | DATE | |
|-----------------------|---|------|----------|-----|
| PATENT INFORMATION: | US 5461143 | | 19951024 | <-- |
| APPLICATION INFO.: | US 1994-219242 | | 19940329 | (8) |
| RELATED APPLN. INFO.: | Continuation of Ser. No. US 1992-889652, filed on 26 May 1992, now abandoned which is a continuation-in-part of Ser. No. US 1992-852409, filed on 16 Mar 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-738211, filed on 30 Jul 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-670701, filed on 18 Mar 1991, now patented, Pat. No. US 5278299 And a continuation-in-part of Ser. No. US 1991-707600, filed on 30 May 1991, now abandoned | | | |
| DOCUMENT TYPE: | Utility | | | |
| FILE SEGMENT: | Granted | | | |
| PRIMARY EXAMINER: | Robinson, Douglas W. | | | |
| ASSISTANT EXAMINER: | Fonda, Kathleen Kahler | | | |
| LEGAL REPRESENTATIVE: | Welsh & Katz, Ltd. | | | |
| NUMBER OF CLAIMS: | 11 | | | |
| EXEMPLARY CLAIM: | 1 | | | |
| NUMBER OF DRAWINGS: | 3 Drawing Figure(s); 3 Drawing Page(s) | | | |
| LINE COUNT: | 3735 | | | |
| AB | Oligosaccharide compounds that are substrates and inhibitors of glycosyltransferase and glycosidase enzymes and compositions containing such compounds are disclosed. A method of glycosylation is also disclosed. An E. coli transformed with phagemid CMPSIL-1, which phagemid comprises a gene for a modified CMP-sialic acid synthetase enzyme, which transformed E. coli has the ATCC accession No. 68531 is also provided. | | | |

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FILE 'HCAPLUS' ENTERED AT 18:37:34 ON 14 AUG 2001

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E WO9936555/PN 25
SET NOTICE 1000 SEARCH
L1 1 SEA ABB=ON PLU=ON WO9936555/PN
SET NOTICE LOGIN SEARCH
SET LINE LOGIN
SET DETAIL LOGIN
D IALL 1

FILE 'HCAPLUS' ENTERED AT 18:41:40 ON 14 AUG 2001

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E E3+ALL
E PROTEINS, SPECIFIC OR CLASS (L) F/CT
E E3+ALL
L2 30992 SEA ABB=ON PLU=ON FUSION PROTEIN# OR CHIMER? PROTEIN# OR
(PROTEINS (L) CHIMERIC) OR (PROTEIN# (L) FUSION PRODUCT#)
E GLYCOSYLTRANSFERASE/CT
E E3+ALL
L3 3206 SEA ABB=ON PLU=ON GLYCOSYLTRANSFERASE#
L4 37 SEA ABB=ON PLU=ON L2 (L) L3
L5 20 SEA ABB=ON PLU=ON L4 AND PD<19971118
D IBIB AB 1-20
L6 388115 SEA ABB=ON PLU=ON (SACCHARIDE# OR MONOSACCHARIDE# OR
OLIGOSACCHARIDE# OR CARBOHYDRATE# OR POLYSACCHARIDE# OR
SUGAR#)
L7 24389 SEA ABB=ON PLU=ON L6 (L) PREP/RL
L8 1 SEA ABB=ON PLU=ON L7 (L) L2 (L) L3

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L5 ANSWER 1 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:706981 HCAPLUS

DOCUMENT NUMBER: 128:46181

TITLE: .beta.1,4 N-acetylgalactosaminyltransferase (GM2/GD2/GA2 synthase) forms homodimers in the endoplasmic reticulum: a strategy to test for dimerization of Golgi membrane proteins

AUTHOR(S): Zhu, Guofen; Jaskiewicz, Ewa; Bassi, Rosaria; Darling, Douglas S.; Young, William W., Jr.

CORPORATE SOURCE: Departments of Biological and Biophysical Sciences and Biochemistry, Schools of Dentistry and Medicine, University of Louisville, Louisville, KY, 40292, USA

SOURCE: Glycobiology (1997), 7(7), 987-996

CODEN: GLYCE3; ISSN: 0959-6658

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Many Golgi membrane-bound **glycosyltransferases** exist as intermol. disulfide bonded species, some of which have been demonstrated to be homodimers. Evidence for homodimer formation has come primarily from radiation inactivation expts. We utilized an alternative strategy to test for homodimer formation of the cloned .beta.1,4 N-acetylgalactosaminyltransferase (GalNAcT) responsible for synthesis of the glycosphingolipids GM2, GD2, and GA2. We stably transfected CHO cells with myc epitope-tagged GalNAcT, which localizes primarily to the Golgi, and a hemagglutinin (HA) epitope-tagged GalNAcT **fusion protein** in which the cytoplasmic domain of GalNAcT was replaced by an ER retention signal. We then sought evidence for dimer formation between the two forms of GalNAcT. Immunopptn. with anti-myc or anti-HA co-immunopptd. the HA-tagged form or the myc-tagged form, resp., providing evidence for the phys. assocn. of the two forms of GalNAcT. As a result of this assocn., GalNAcT/myc increased in the ER as demonstrated by Western blots and immunofluorescence. The rapid formation of dimers provided further evidence for dimer formation occurring in the ER. In summary, these results demonstrate that GalNAcT forms homodimers as a result of intermol. disulfide bond formation in the ER. Furthermore, this ER motif strategy is potentially useful for demonstrating homodimer formation of other Golgi enzymes.

L5 ANSWER 2 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:448100 HCAPLUS

DOCUMENT NUMBER: 127:61642

TITLE: expression of genes for mammalian glycosyltransferases for alteration of patterns of protein glycosidation in fungal hosts

PATENT ASSIGNEE(S): Ernst, Joachim, Germany

SOURCE: Ger. Offen., 7 pp.

CODEN: GWXXBX

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|-------------|------|----------|------------------|--------------|
| DE 19546680 | A1 | 19970619 | DE 1995-19546680 | 19951214 <-- |

AB A method of altering the patterns of glycosidation of **proteins** manufd. in a yeast host by expression of genes for mammalian **glycosyltransferases** is described. The mammalian genes may included in **chimeric** genes with those for fungal **glycosyltransferase**, e.g to ensure targetting of the enzyme to the correct organelle. A **chimeric** gene for a **fusion**

protein of a human galactosyltransferase and a yeast mannosyltransferase using the anchor domain of the mannosyltransferase was constructed by std. methods and expressed in *Saccharomyces cerevisiae* using the GAL10 promoter. The galactosyltransferase manufd. in yeast had the same Km (44 .mu.M) as the human form for UDP-galactose and had a Vmax of 0.184 vs. 0.7 .mu.mol/min/mg protein.

L5 ANSWER 3 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:312571 HCAPLUS
DOCUMENT NUMBER: 126:316364
TITLE: Production of antibody to detect and purify cyclodextrin glycosyltransferase and over-production of its soluble proteins using gene fusion and controlled environment (starch, CGT)
AUTHOR(S): Han, Nam Soo
CORPORATE SOURCE: Purdue Univ., West Lafayette, IN, USA
SOURCE: (1996) 171 pp. Avail.: Univ. Microfilms Int., Order No. DA9713519
From: Diss. Abstr. Int., B 1997, 57(11), 6676
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable

L5 ANSWER 4 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:281159 HCAPLUS
DOCUMENT NUMBER: 126:260139
TITLE: Human **glycosyltransferase** or fucosyltransferase **fusion proteins**, **chimeric** genes encoding them, and expression in host cell
INVENTOR(S): Lowe, John B.; Legault, Daniel J.
PATENT ASSIGNEE(S): Regents of the University of Michigan, USA
SOURCE: PCT Int. Appl., 329 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|--------------|
| WO 9709421 | A1 | 19970313 | WO 1996-US13816 | 19960906 <-- |
| W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM | | | | |
| US 5770420 | A | 19980623 | US 1995-525058 | 19950908 |
| AU 9669035 | A1 | 19970327 | AU 1996-69035 | 19960906 <-- |
| PRIORITY APPLN. INFO.: | | | US 1995-525058 | 19950908 |
| | | | WO 1996-US13816 | 19960906 |

AB A method for isolating a gene, comprising: (1) isolating a cell possessing a post-translational characteristic of interest, said post-translational characteristic being the presence of a membrane-bound oligosaccharide or polysaccharide of interest on the surface of said cell, the presence of a sol. oligosaccharide or polysaccharide of interest in an ext. of said cell, or the presence of a particularly **glycosyltransferase** activity in an ext. of said cell; (2) creating a genetic library of either cDNA or genomic DNA from the genetic material of said isolated cell; (3) transforming host cells with said genetic library; and (4) screening said transformed host cells for a host cell contg. said post-translational characteristic, thereby obtaining a cell contg. said gene, is disclosed.

The method can be used to obtain genes encoding **glycosyltransferases**. Esp. plasmid vectors for expression of fucosyltransferase **fusion proteins** are described. Many alternative fucosyltransferase III-fucosyltransferase TVI **chimeric** genes are included.

L5 ANSWER 5 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:175169 HCAPLUS

DOCUMENT NUMBER: 126:168883

TITLE: An in vivo approach for the identification of acceptor sites for O-glycosyltransferases: Motifs for the addition of O-GlcNAc in Dictyostelium discoideum

AUTHOR(S): Jung, Eva; Gooley, Andrew A.; Packer, Nicolle H.; Slade, Martin B.; Williams, Keith L.; Ditttrich, Werner

CORPORATE SOURCE: MUCAB (Macquarie University Center for Analytical Biotechnology) School of Biological Sciences, Macquarie University, Sydney, 2109, Australia

SOURCE: Biochemistry (1997), 36(13), 4034-4040

CODEN: BICHAW; ISSN: 0006-2960

PUBLISHER: American Chemical Society

DOCUMENT TYPE: Journal

LANGUAGE: English

AB To identify and analyze acceptor sequences for O-glycosylation, we have developed an in vivo system expressing short peptides as glutathione S-transferase fusion proteins in the eukaryotic host Dictyostelium discoideum. Using this approach, we show that a short peptide motif (PTVTPT), present in the D. discoideum cell-surface glycoprotein PsA, is sufficient as a signal for O-glycosylation, even when fused to a heterologous protein. Monosaccharide anal. and solid-phase protein sequencing showed that the modification is a single N-acetylglucosamine attached to threonine residues. This was further confirmed by electrospray-mass spectrometry. The O-linked glycosylation of both this peptide and authentic PsA presents the modB-dependent carbohydrate-specific epitope identified by the monoclonal antibody MUD50. Substitution of threonine by serine residues in this peptide also yields a glycosylated fusion protein which is modified with single N-acetylglucosamine residues, but not all of the serines are glycosylated.

L5 ANSWER 6 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:137084 HCAPLUS

DOCUMENT NUMBER: 126:234768

TITLE: Golgi localization of glycosyltransferases: more questions than answers

AUTHOR(S): Colley, Karen J.

CORPORATE SOURCE: College of Medicine, University of Illinois, Chicago, IL, 60612, USA

SOURCE: Glycobiology (1997), 7(1), 1-13

CODEN: GLYCE3; ISSN: 0959-6658

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review with .apprx.55 refs. The structures of cellular oligosaccharides are detd. by a series of processing reactions catalyzed by Golgi glycosidases and **glycosyltransferases**. While there are subtle cell type differences in Golgi enzyme subcompartmentation, in general, glycosylation enzymes are localized within the Golgi cisternae in the same sequence in which they act to modify oligosaccharide substrates. The possibility that this enzyme subcompartmentation may control the types of oligosaccharides expressed by a cell has led to an interest in the signals and mechanisms directing enzyme localization in the Golgi cisternae. All glycosidases and **glycosyltransferases** characterized thus far have very little sequence homol. that might suggest a common Golgi retention signal, but they do share a similar domain structure. They are all type II transmembrane **proteins** consisting of an amino

terminal cytoplasmic tail, a signal anchor transmembrane domain, a stem region, and a large luminal catalytic domain. Their lack of sequence homol. suggests that these **proteins'** Golgi retention signals are not linear amino acid sequences, but most likely involve general characteristics or conformations or larger protein domains. The peptide sequences required for Golgi retention of the N-acetylglucosaminyltransferase I (GlcNAcTI), .beta.1,4-galactosyltransferase (GalT) and .alpha.2,6-sialyltransferase (ST) have been extensively studied. To do this, researchers created mutant and **chimeric proteins**, expressed these in tissue culture cells, and localized these **proteins** using immunofluorescence microscopy. The cell surface expression of deletion mutants suggested that the deleted sequences were necessary for Golgi retention. Then, if these sequences were fused to a non-Golgi reporter protein and this **chimeric** or hybrid protein was retained in the Golgi, then these sequences were also sufficient for Golgi retention. Due to differences in reporter **proteins** used to construct these **chimeric proteins**, different cell types used for protein expression, different levels of protein expression, and different methods of cell surface protein detection, these expts. have led to somewhat confusing results. However, in general, it appears that the GalT relies primarily on its transmembrane domain for Golgi retention, while the GlcNAcTI and ST have requirements for their transmembrane regions, sequences flanking these regions, and luminal stem sequences. Based on these results, two potential Golgi retention mechanisms have been proposed and are now being tested. The observation that **glycosyltransferase** transmembrane domains are frequently sufficient for Golgi retention has led to the first of these models, the bilayer thickness model. This model proposes that the shorter transmembrane domains of Golgi **proteins** prevent them from entering cholesterol-rich transport vesicles destined for the plasma membrane, and that this leads to Golgi retention. The second of these models is supported by the role of multiple protein domains in the Golgi retention of some **proteins**. This model, the oligomerization/kinetics recognition model of Golgi retention, proposes that the formation of insol. protein homo-oligomers or very large hetero-oligomers prevents protein movement into transport vesicles destined for later compartments. Initial work suggests that the bilayer thickness mechanism may play a role in the retention of some Golgi retained **proteins**; however, it is not the sole retention mechanism. Other evidence suggests that an oligomerization/kinetics recognition mechanism may be more common, but definitive proof for its general use in Golgi protein retention is lacking. More research is required to further elucidate the sequences and particularly the mechanisms of Golgi retention. In the future, the authors hope to be able to explain the cell type differences in glycosylation enzyme Golgi subcompartmentation, the different sequence requirements for the Golgi retention of the same enzyme in various cell types, and whether differences in glycosylation enzyme Golgi subcompartmentation change the types of oligosaccharides made by a cell.

L5 ANSWER 7 OF 20 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:53962 HCAPLUS
DOCUMENT NUMBER: 126:72038
TITLE: Expression of glycosyltransferase in Aspergillus with
increased secretion
INVENTOR(S): Kadoma, Katherine H.; Ward, Michael
PATENT ASSIGNEE(S): Genencor International, Inc., USA
SOURCE: PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|--------------|
| WO 9636718 | A1 | 19961121 | WO 1996-US7171 | 19960516 <-- |
| W: CA, FI, JP, MX | | | | |
| RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| CA 2221271 | AA | 19961121 | CA 1996-2221271 | 19960516 <-- |
| EP 826054 | A1 | 19980304 | EP 1996-920281 | 19960516 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI | | | | |
| PRIORITY APPLN. INFO.: | | | US 1995-443954 | 19950518 |
| | | | WO 1996-US7171 | 19960516 |

AB A method for the expression of **glycosyltransferase** in *Aspergillus* with increased level of secretion is described. The method consists of expression of a chimeric DNA comprised from 5'-terminus of (1) a signal sequence; (2) a DNA sequence encoding a secreted protein of a filamentous fungi, preferably *Aspergillus*; (3) a DNA sequence encoding a cleavable linker; and (4) a DNA sequence encoding a **glycosyltransferase**. Prepn. of a **fusion protein** consisting of a truncated form of .alpha.-2,3-sialyltransferase and the entire glucoamylase and expression of the **fusion protein** in *Aspergillus niger* var. *awamori* were demonstrated.

L5 ANSWER 8 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:40940 HCAPLUS.
DOCUMENT NUMBER: 126:85444
TITLE: Cloning of the gene for monogalactosyldiacylglycerol synthase and its evolutionary origin
AUTHOR(S): Shimojima, Mie; Ohta, Hiroyuki; Iwamatsu, Akihiro; Masuda, Tatsuru; Shioi, Yuzo; Takamiya, Ken-ichiro
CORPORATE SOURCE: Fac. Biosci. Biotechnol., Tokyo Inst. Technol., Yokohama, 226, Japan
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1997), 94(1), 333-337
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Monogalactosyldiacylglycerol (MGDG) synthase (UDPGalactose:1,2-diacylglycerol 3-.beta.-D-galactosyltransferase; EC 2.4.1.46) catalyzes formation of MGDG, a major structural lipid of chloroplast. We cloned a cDNA for the synthase from cucumber cDNA library. The full-length cDNA clone was 2142 bp, and it contains a 1575-bp open reading frame encoding 525 aa. The open reading frame consists of the regions for a mature protein (422 aa; Mr of 46,552) and transit peptide to chloroplast (103 aa). Although the mol. wt. of mature protein region matched that purified from cucumber cotyledons, it was quite different from those purified from spinach (.+- .20 kDa) reported by other groups. The mature region of the protein was expressed in *Escherichia coli* as a **fusion protein** with glutathione S-transferase. The expression in *E. coli* showed that the protein catalyzed MGDG synthesis very efficiently. Therefore, we concluded that the cDNA encodes MGDG synthase in cucumber. In addn., the deduced amino acid sequence of the MGDG synthase cDNA showed homol. with MurG of *Bacillus subtilis* and *E. coli*, which encode a **glycosyltransferase** catalyzing the last step of peptidoglycan synthesis in bacteria. This sequence homol. implies that the machinery of chloroplast membrane biosynthesis is evolutionarily derived from that of cell wall biosynthesis in bacteria. This is consistent with the endosymbiotic hypothesis of chloroplast formation.

L5 ANSWER 9 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:7801 HCAPLUS
DOCUMENT NUMBER: 126:128654
TITLE: Switching amino-terminal cytoplasmic domains of .alpha.(1,2)fucosyltransferase and .alpha.(1,3)galactosyltransferase alters the

expression of H substance and Gal.alpha.(1,3)Gal
AUTHOR(S): Osman, Narin; McKenzie, Ian F. C.; Mouhtouris, Effie;
Sandrin, Mauro S.
CORPORATE SOURCE: Austin Res. Inst., Austin Hosp., Heidelberg, 3084,
Australia
SOURCE: J. Biol. Chem. (1996), 271(51), 33105-33109
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular
Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB When .alpha.(1,2)fucosyltransferase cDNA is expressed in cells that normally express large amts. of the terminal carbohydrate Gal.alpha.(1,3)Gal, and therefore the .alpha.(1,3)galactosyltransferase (GT), the Gal.alpha.(1,3)Gal almost disappears, indicating that the presence of the .alpha.(1,2)fucosyltransferase (HT) gene/enzyme alters the synthesis of Gal.alpha.(1,3)Gal. A possible mechanism to account for these findings is enzyme location within the Golgi app. We examd. the effect of Golgi localization by exchanging the cytoplasmic tails of HT and GT; if Golgi targeting signals are contained within the cytoplasmic tail sequences of these enzymes then a tail switch would permit GT first access to the substrate and thereby reverse the obsd. dominance of HT. Two **chimeric glycosyltransferase proteins** were constructed and compared with the normal **glycosyltransferases** after transfection into COS cells. The **chimeric** enzymes showed Km values and cell surface carbohydrate expression comparable with normal **glycosyltransferases**. Coexpression of the two **chimeric glycosyltransferases** resulted in cell surface expression of Gal.alpha.(1,3)Gal, and virtually no HT product was expressed. Thus the cytoplasmic tail of HT dets. the temporal order of action, and therefore dominance, of these two enzymes.

L5 ANSWER 10 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:599693 HCAPLUS
DOCUMENT NUMBER: 125:271382
TITLE: Modification of the cytoplasmic domain affects the subcellular localization of Golgi glycosyltransferases
AUTHOR(S): Yang, Wei; Pepperkok, Rainer; Bender, Patrick; Kreis, Thomas E.; Storrie, Brian
CORPORATE SOURCE: Virginia Polytechnic Institute, State University, Blacksburg, VA, 24061-0308, USA
SOURCE: Eur. J. Cell Biol. (1996), 71(1), 53-61
CODEN: EJCBND; ISSN: 0171-9335
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Our goal was to engineer a Golgi **glycosyltransferase** epitope-tagged on its cytoplasmically exposed, short, N-terminal domain that gave normal subcellular localization. Partial replacement of the cytoplasmic tail of human .alpha.-2,6-sialyltransferase (SialylT) with the neg. charged myc or FLAG epitope resulted in almost complete mislocalization of the chimera expressed in Vero cells. A granular cytoplasmic staining pattern was seen by immunofluorescence. Spacing the neg. charged residues progressively outward from the neg. N-terminus resulted in increasingly more normal localization of myc or FLAG-tagged protein to a juxtanuclear Golgi-like distribution. Substitution of a neutrally charged VSV-G sequence for these tags resulted in normal localization of the chimera to the juxtanuclear Golgi region. Insertion of the myc epitope within the N-terminal domain of the short form of bovine .beta.-1,4-galactosyltransferase (GalT) gave a **chimeric protein** that mislocalized in BHK cells. No signal was detected with a monoclonal anti-epitope antibody indicating that the myc epitope was masked. Placement of myc or FLAG epitopes at the NH2-terminus of human N-acetylglucosaminyltransferase I (GlcNAc-T) resulted in **chimeric proteins** that in Vero cells displayed little

Golgi localization. We conclude that positioning of neg. charge, in particular, close to the membrane, typically produces a failure of type II Golgi **glycosyltransferases** to exit the endoplasmic reticulum/cis-Golgi network, presumably due to quality control mechanisms. These **proteins** may be successfully epitope-tagged on their N-terminal domain either using a neutral or pos. charged sequence or spacing any neg. charged sequence out from the membrane.

L5 ANSWER 11 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:351599 HCAPLUS
DOCUMENT NUMBER: 125:51989
TITLE: Immunodetection of .alpha.1-3 fucosyltransferase (FucT-V)
AUTHOR(S): Borsig, Lubor; Kleene, Ralf; Dinter, Andre; Berger, Eric G.
CORPORATE SOURCE: Institute Physiology, University Zurich, Zurich, Switz.
SOURCE: Eur. J. Cell Biol. (1996), 70(1), 42-53
CODEN: EJCBDN; ISSN: 0171-9335
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The fucosyltransferases constitute a family of **glycosyltransferases** incorporating fucose residues into glycoprotein or glycolipid glycans. They afford one of the possible termination steps of glycoconjugate biosynthesis creating the sialyl Lewisx or sialyl Lewisx determinant, which play an important role in cell-cell interaction. Whereas cDNA, chromosomal localization, and kinetic properties of a no. of fucosyltransferases are known, immunocytochem. localization and trafficking studies have been delayed because of the lack of specific antibodies due to the pronounced homol. of .alpha.1,3-fucosyltransferases III, V, and VI. Here, the development and characterization of monospecific polyclonal antibodies to .alpha.1-3-fucosyltransferase V (FucT-V) and their application for immunodetection in transfected cells are reported. Antisera against FucT-V were raised in 2 different ways: 1st by producing a **fusion protein** .beta.-galactosidase-FucT-V in Escherichia coli, and by synthesizing a peptide stretch specific for FucT-V. Polyclonal antisera were raised against each of both antigens and characterized by ELISA, neutralization of activity, immunoblotting, immunofluorescence, and immunopptn. of metabolically labeled COS cells, transiently transfected with cDNA encoding FucT-V. Both antibodies recognized only FucT-V. No crossreactivity to FucT-III or FucT-VI was obsd. FucT-V was localized mainly to the Golgi app. by colocalization with .beta.1,4-galactosyltransferase, and to the cell surface of COS, CHO, and HeLa cells. Expression of FucT-V in COS cells revealed 3 isoforms of 58, 53 and 50 kDa, resp. These size differences arose by posttranslational modifications, as shown by pulse-chase expts. The results indicate that .alpha.1-3-fucosyltransferase is a Golgi-assocd. enzyme and suggest its possible occurrence on the cell surface.

L5 ANSWER 12 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:180589 HCAPLUS
DOCUMENT NUMBER: 124:226128
TITLE: Localization of a yeast early Golgi mannosyltransferase, Ochlp, involves retrograde transport
AUTHOR(S): Harris, Sandra L.; Waters, M. Gerard
CORPORATE SOURCE: Dep. Molecular Biology, Princeton Univ., Princeton, NJ, 08544, USA
SOURCE: J. Cell Biol. (1996), 132(6), 985-98
CODEN: JCLBA3; ISSN: 0021-9525
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To analyze the mechanism of integral membrane protein localization in the

early Golgi app. of *Saccharomyces cerevisiae*, the authors have used Ochlp, a cis-Golgi mannosyltransferase. A series of influenza virus hemagglutinin (HA) epitope-tagged **fusion proteins** was constructed in which invertase is appended to the Golgi-luminal carboxy terminus of full-length Ochlp. Several constructs included a Kex2p cleavage site between the Ochlp and invertase moieties to monitor transit to the Kex2p-contg. TGN. Cells expressing an Ochlp-invertase fusion do not secrete invertase, but those expressing an Ochlp-Kex2p site-invertase **fusion protein** secrete high levels of invertase in a Kex2p-dependent manner. The Ochlp-Kex2p site-invertase **fusion protein** is cleaved with a half-time of 5 min, and the process proceeds to completion. Before cleavage the protein receives glycosyl modifications indicative of passage through the medial- and trans-Golgi; therefore, cleavage occurs after ordered anterograde transport through the Golgi to the TGN. Transit to distal compartments is not induced by the invertase moiety, since noninvertase fusion constructs encounter the same **glycosyltransferases** and Kex2p as well. The Ochlp-HA moiety, irresp. of whether it is generated by cleavage of the **fusion protein** in the TGN or synthesized de novo, is degraded with a half-time of about 60 min. Thus, the half-time of degrdn. is 12-fold longer than the time required to reach the TGN. At steady state, de novo-synthesized and TGN-generated HA epitope-tagged Ochlp reside in a compartment with a buoyant d. identical to that of wild-type Ochlp and distinct from that of the vacuole or the TGN. Finally, ochl null cells that express an Ochlp fusion construct known to rapidly encounter the TGN glycosylate invertase to the same extent as wild-type cells, indicating that they have phenotypically wild-type Ochlp activity. These results led the authors to propose a model for Ochlp-HA localization that involves movement to distal compartments, at least as far as the TGN, followed by retrieval to the cis compartment, presumably by vesicular transport.

L5 ANSWER 13 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:117724 HCAPLUS

DOCUMENT NUMBER: 124:166738

TITLE: Golgi localization and in vivo activity of a mammalian glycosyltransferase (human .beta.1,4-galactosyltransferase) in yeast

AUTHOR(S): Schwientek, Tilo; Narimatsu, Hisashi; Ernst, Joachim F.

CORPORATE SOURCE: Inst. Mikrobiol., Heinrich-Heine-Univ. Duesseldorf, Duesseldorf, D-40225, Germany

SOURCE: J. Biol. Chem. (1996), 271(7), 3398-405
CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Gene fusions encoding the membrane anchor region of yeast .alpha.1,2-mannosyltransferase (Mntlp) fused to human .beta.1,4-galactosyltransferase (Gal-Tf) were constructed and expressed in the yeast *Saccharomyces cerevisiae*. **Fusion proteins** contg. 82 or only 36 N-terminal residues of Mntlp were produced and quant. N-glycosylated; glycosyl chains were shown to contain .alpha.1,6-, but not .alpha.1,3-mannose determinants, a structure typical for an early Golgi compartment. A final Golgi localization of both fusions was confirmed by sucrose gradient fractionations, in which Gal-Tf activity cofractionated with Golgi Mntlp activity, as well as by immunocytol. localization expts. using a monoclonal anti-Gal-Tf antibody. In an in vitro Gal-Tf enzymic assay the Mnt1/Gal-Tf fusion and sol. human Gal-Tf had comparable Km values for UDP-Gal (about 45 .mu.M). To demonstrate in vivo activity of the Mnt1/Gal-Tf fusion the encoding plasmids were transformed in an algi mutant, which at the non-permissive temp. transfers short (GlcNAc)2 glycosyl chains to proteins. Using specific lectins the addn. of galactose to several yeast proteins in transformants could be detected. These results demonstrate that Gal-Tf, a mammalian **glycosyltransferase**, is functional in the mol. environment of the

yeast Golgi, indicating conservation between yeast and human cells. The in vivo function of human Gal-Tf indicates that the yeast Golgi is accessible for UDP-Gal and suggests strategies for the construction of yeast strains, in which desired glycoforms of heterologous proteins are produced.

L5 ANSWER 14 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:81591 HCAPLUS

DOCUMENT NUMBER: 124:127138

TITLE: Glucan-binding proteins, especially **glycosyltransferase**, and use of **fusion proteins** to prevent dental plaque and teeth staining

INVENTOR(S): Kuramitsu, Howard Kikuo; Schilling, Kurt Matthew

PATENT ASSIGNEE(S): Unilever NV, Neth.; Unilever PLC

SOURCE: PCT Int. Appl., 44 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|--------------|
| WO 9531556 | A1 | 19951123 | WO 1995-GB1070 | 19950511 <-- |
| W: JP | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| EP 759081 | A1 | 19970226 | EP 1995-918091 | 19950511 <-- |
| R: DE, FR, GB, IT | | | | |
| JP 10500127 | T2 | 19980106 | JP 1995-529437 | 19950511 |
| PRIORITY APPLN. INFO.: | | | GB 1994-9387 | 19940511 |
| | | | WO 1995-GB1070 | 19950511 |

AB Polypeptides with specific binding affinity for glucan - esp. the glucan-binding domain of glycosyl transferase enzyme - is utilized in a compn. for oral care. The polypeptide may block the binding sites in dental plaque where glycosyl transferase would bind and generate more plaque, or it may be conjugated to - and provide targeted delivery of - an antiplaque or antistain agent.

L5 ANSWER 15 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:589699 HCAPLUS

DOCUMENT NUMBER: 123:193332

TITLE: A second ABC transporter is involved in oleandomycin resistance and its secretion by Streptomyces antibioticus

AUTHOR(S): Olano, Carlos; Rodriguez, Ana Maria; Mendez, Carmen; Salas, Jose A.

CORPORATE SOURCE: Inst. Univ. Biotechnology, Univ. Oviedo, Oviedo, 33006, Spain

SOURCE: Mol. Microbiol. (1995), 16(2), 333-43

CODEN: MOMIEE; ISSN: 0950-382X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A 3.2-kb SstI-SphI DNA fragment of S. antibioticus, an oleandomycin producer, conferring resistance to oleandomycin was sequenced and found to contain an open reading frame of 1710 bp (oleB). Its deduced gene product (OleB) showed a high degree of similarity with other proteins belonging to the ABC-transporter superfamily, including the gene product of another oleandomycin-resistance gene (OleC). The OleB protein contains 2 ATP-binding domains, each of .apprx.200 amino acids in length, and no hydrophobic transmembrane regions. Functional anal. of the oleB gene was carried out by deleting specific regions of the gene and assaying for oleandomycin resistance. These expts. showed that either the 1st or the 2nd half of the gene contg. only 1 ATP-binding domain was sufficient to

confer resistance to oleandomycin. The gene *oleB* was expressed in *Escherichia coli* fused to a maltose-binding protein (MBP) using the pMal-c2 vector. The MBP-OleB hybrid protein was purified by affinity chromatog. on an amylose resin and polyclonal antibodies were raised against the **fusion protein**. These were used to monitor the biosynthesis and phys. location of OleB during growth. By Western anal., the OleB protein was detected both in the sol. and in the membrane fraction and its synthesis paralleled oleandomycin biosynthesis. A *Streptomyces albus* strain, contg. both a **glycosyltransferase** (OleD) able to inactivate oleandomycin and the OleB protein, was capable of glycosylating oleandomycin and secreting the inactive glycosylated mol. It is proposed that OleB constitutes the secretion system by which oleandomycin or its inactive glycosylated form could be secreted by *S. antibioticus*.

L5 ANSWER 16 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:574279 HCAPLUS

DOCUMENT NUMBER: 123:140108

TITLE: Cell type differences in Golgi retention signals for transmembrane proteins

AUTHOR(S): Tang, Bor Luen; Low, Seng Hui; Wong, Siew Heng; Hong, Wanjin

CORPORATE SOURCE: Institute of Molecular and Cell Biology, National University of Singapore, Singapore, 0511, Singapore

SOURCE: Eur. J. Cell Biol. (1995), 66(4), 365-74

CODEN: EJCBDN; ISSN: 0171-9335

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The transmembrane domain of Golgi resident **proteins** such as .beta.-galactoside .alpha.2,6-sialyltransferase (ST) and N-acetylglucosaminyltransferase 1 (NT) contain a Golgi retention signal which confers Golgi retention to reporter **proteins** appended to them in the appropriate context. Thus, chimeras of the cell surface protein dipeptidyl peptidase IV contg. the transmembrane domain of ST and NT are retained in the Golgi app. in MDCK and COS cells, as assessed by indirect immunofluorescence microscopy. Transfection of these **chimeric** constructs into CHO cells, however, results in their transport to vesicular structures which do not colocalize with that of an endogenous Golgi marker, mannosidase II. Furthermore, the staining pattern of these structures are not affected by brefeldin A. Biochem. anal. of the transgene products in pulse-chase expts. revealed that the **chimeric proteins** eventually become resistant to endoglycosidase H, suggesting that they are transported beyond the medial Golgi and therefore the vesicular structures are likely to be post-Golgi. The vesicular structures colocalized well with a lysosomal marker, cathepsin D, and also with internalized FITC-dextran chased into the lysosomal compartment. Monitoring the cell surface appearance of the **chimeric protein** suggests that the majority is transported directly to the lysosomal compartment. Golgi retention can be completely restored for ST and improved for NT by the inclusion of sequences flanking the transmembrane domain. Our results reflect cell type differences in the interpretation of the transmembrane domain Golgi retention signal, established that general Golgi retention of type II **glycosyltransferases** requires the hydrophilic flanking sequence as well as the transmembrane domain, and demonstrate that **proteins** which escape Golgi retention may be channeled to the lysosomal pathway.

L5 ANSWER 17 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:440702 HCAPLUS

DOCUMENT NUMBER: 122:182984

TITLE: Golgi localization in yeast is mediated by the membrane anchor region of rat liver sialyltransferase

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J. Biol. Chem. (1995), 270(10), 5483-9
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To investigate the function of the membrane anchor region of a mammalian **glycosyltransferase** in yeast we constructed a fusion gene that encodes the 34 amino-terminal residues of rat liver .beta.-galactoside .alpha.-2,6-sialyl-transferase (EC 2.4.99.1) (ST) fused to the mature form of yeast invertase. Transformants of *Saccharomyces cerevisiae* expressing the fusion gene produced an intracellular heterogeneously N-glycosylated **fusion protein** of intermediate mol. wt. between the core and fully extended N-glycosylated form of invertase, suggesting a post-endoplasmic reticulum (ER) localization. In two types of cell fractionation using sucrose d. gradients the ST-invertase **fusion protein** cofractionated with Golgi marker proteins, whereas a minor fraction (about 30%) comigrated with a vacuolar marker; ST-invertase was not detected in other cell fractions, including the ER and the plasma membrane. Consistent with Golgi localization, about 70% of the total amt. of the ST-invertase fusion was immunoprecipitated with an antibody directed against .alpha.-1,6-mannose linkages. The results demonstrate that the membrane anchor region of a mammalian type II **glycosyltransferase** is able to target a protein to the secretory pathway and to a Golgi compartment of the yeast *S. cerevisiae*, indicating conservation of targeting mechanisms between higher and lower eukaryotes. Since typical yeast Golgi localization signals are missing in the ST-membrane anchor region the results also suggest that yeast as mammalian cells utilize diverse mechanisms to direct proteins to the Golgi.

L5 ANSWER 18 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1994:128404 HCAPLUS
DOCUMENT NUMBER: 120:128404
TITLE: Expression of deletion constructs of bovine .beta.-1,4-galactosyltransferase in *Escherichia coli*: Importance of Cys134 for its activity
AUTHOR(S): Boeggeman, Elizabeth E.; Balaji, Petety V.; Sethi, Navin; Masibay, Arni S.; Qasba, Pradman K.
CORPORATE SOURCE: Lab. Math. Biol., Natl. Cancer Inst., Bethesda, MD, 20892, USA
SOURCE: Protein Eng. (1993), 6(7), 779-85
CODEN: PRENE9; ISSN: 0269-2139
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Bovine acetylglucosamine .beta.-1,4-galactosyltransferase (EC 2.4.1.90) (I) belongs to the **glycosyltransferase** family and as such shares a general topology: an N-terminal cytoplasmic tail, a signal anchor followed by a stem region, and a catalytic domain at the C-terminal end of the protein. CDNA constructs of the N-terminal-deleted forms of I were prepared in a pGEX-2T vector and expressed in *E. coli* as glutathione S-transferase (GST) **fusion proteins**. Recombinant proteins accumulated within inclusion bodies as insoluble aggregates that were solubilized in 5M guanidine-HCl and required an oxido-shuffling reagent for regeneration of the enzyme activity. Recombinant I, devoid of the GST domain, had 30-85% of the specific activity of bovine milk I with apparent K_m values for N-acetylglucosamine and UDP-galactose similar to those of milk I. Deletion analyses showed that both the I and lactose synthetase activities remained intact even in the absence of the first 129 residues (pGT-d129). The activities were lost when either deletions extended up to residue 142 (pGT-d142) or when Cys-134 was mutated to Ser (pGT-d129C134S). These results suggested that the formation of a disulfide bond involving Cys-134 holds the protein in a conformation that is required for enzymic activity.

L5 ANSWER 19 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1993:646599 HCAPLUS
DOCUMENT NUMBER: 119:246599
TITLE: Specific sequences in the signal anchor of the .beta.-galactoside .alpha.-2,6-sialyltransferase are not essential for Golgi localization. Membrane flanking sequences may specify Golgi retention
AUTHOR(S): Dahdal, Refka Y.; Colley, Karen J.
CORPORATE SOURCE: Coll. Med., Univ. Illinois, Chicago, IL, 60612, USA
SOURCE: J. Biol. Chem. (1993), 268(35), 26310-19
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The .beta.-galactoside .alpha.-2,6-sialyltransferase is a trans Golgi/trans Golgi network **glycosyltransferase** which adds sialic acid residues to Asn-linked oligosaccharides of glycoproteins. Previous results suggested that the sialyltransferase stem and signal anchor including flanking sequences may be 2 independent Golgi retention regions. However, other expts. demonstrated that the sequence of the signal anchor itself was not important. To investigate whether the sialyltransferase signal anchor was necessary and sufficient for Golgi retention, several mutant and **chimeric proteins** were expressed and localized in Cos-1 and CHO cells. The signal anchor and flanking sequences were able to retain the sialyltransferase catalytic domain in the Golgi. However, efficient Golgi retention was still obsd. when the signal anchor was altered or entirely replaced in either the presence or absence of most of the luminal stem region. **Chimeric proteins** consisting of the sialyltransferase cytoplasmic tail and signal anchor fused to the extracellular domains of 2 different cell surface **proteins** demonstrated poor Golgi retention. A significant increase in the Golgi retention of 1 of these chimeras was obsd. when 2 lysines were placed next to the signal anchor on the luminal side. Taken together these results suggest that the sialyltransferase signal anchor is not necessary or sufficient for Golgi retention, rather, appropriately spaced cytoplasmic and luminal flanking sequences are the important elements of the sialyltransferase Golgi retention region.

L5 ANSWER 20 OF 20 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1993:464389 HCAPLUS
DOCUMENT NUMBER: 119:64389
TITLE: Expression cloning of a cDNA encoding UDP-GlcNAc:Gal.beta.1-3-GalNAc-R (GlcNAc to GalNAc) .beta.1-6GlcNAc transferase by gene transfer into CHO cells expressing polyoma large tumor antigen
AUTHOR(S): Bierhuizen, Marti F. A.; Fukuda, Minoru
CORPORATE SOURCE: Cancer Res. Cent., La Jolla Cancer Res. Found., La Jolla, CA, 92037, USA
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1992), 89(19), 9326-30
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A cDNA encoding UDP-GlcNAc:Gal.beta.1-3GalNAc-R (GlcNA to GalNAc) .beta.1-6GlcNAc transferase (EC 2.4.1.102), which forms crit. branches in O-glycans, has been isolated by an expression cloning approach using Chinese hamster ovary (CHO) cells. Increased activity of this enzyme and the concomitant occurrence of the O-glycan core 2 structure [Gal.beta.1-3(GlcNAc.beta.1-6)GalNAc] has been obsd. in a variety of biol. processes, such as T-cell activation and immunodeficiency due to the Wiskott-Aldrich syndrome and AIDS. Since CHO cells do not express this enzyme, CHO cell lines were established to stably express polyoma large tumor (T) antigen, which enables transient expression cloning. Because the antibody used was found to detect most efficiently the oligosaccharide products attached to leukosialin, the CHO cells were also stably transfected with leukosialin cDNA. By using this particular CHO cell

line, a cDNA that encodes a protein detg. the formation of the core 2 structure was isolated from an HL-60 cDNA library. The cDNA sequence predicts a protein with type II membrane topol., as has been found for all other mammalian **glycosyltransferases** cloned to date. The expression of the presumed catalytic domain as a **fusion protein** with the IgG binding domain of protein A permitted unequivocal demonstration that the cDNA encodes the core 2 .beta.-1,6-N-acetylglucosaminyltransferase, the enzyme responsible for the formation of Gal.beta.1-3(GlcNAc.beta.1-6)GalNAc structures. No activity with this enzyme was detected toward the acceptors for the .beta.1-6GlcNAc transferases.